Departement für Nutztiere, Abteilung Ambulanz und Bestandesmedizin
der Vetsuisse-Fakultät Universität Zürich

Direktor: Prof. Dr. Heinrich Bollwein

Predicting fertility in cows with clinical endometritis based on a number of clinical symptoms and examination results

Inaugural-Dissertation

zur Erlangung der Doktorwürde der
Vetsuisse-Fakultät Universität Zürich

vorgelegt von

Martina Ernstberger

Tierärztin
aus Tirschenreuth, Deutschland

genehmigt auf Antrag von

Prof. Dr. Heinrich Bollwein, Referent
PD Dr. Maike Heppelmann, Korreferentin

2018

Meinen Eltern

INDEX

Summary

Vetsuisse-Faculty University of Zurich

Martina Ernstberger

Departement for Farm Animals
Ambulatory Service and Herd Health Medicine
cgeisinger@vetclinics.uzh.ch

Predicting fertility in cows with clinical endometritis based on a number of clinical symptoms and examination results

The objective of this study was to improve prediction of the reproductive performance in cows with clinical endometritis by means of a number of examination results. For this purpose, data from 300 cows were collected. The diagnosis of clinical endometritis at puerperal control 1 (PC 1 = 22-45 days in milk) was made by vaginal discharge scoring with a Metricheck™ device (endometritis score (ES) 1-3). Cow-specific data were collected and detailed general clinical and gynaecological examinations of the genital tract as well as a microbiological investigation of the vaginal discharge were performed. A second puerperal control was performed 21 days later. Puerperal diseases prior to the study, external vaginal discharge (EVD) and the presence of *Trueperella pyogenes* (TP) favored the development of an ES 3. The presence of an ES 3 at PC 1 was in turn associated with impaired fertility (higher number of days to first service, lower conception rate to all services, higher culling rate due to infertility).
In the multivariable statistical model, TP was the decisive factor for the prediction of the pregnancy status at 100, 150 and 200 days in milk, if the bacteriological findings were included besides the remaining examination results. If not, EVD was most decisive of all data taken into account. Therefore, the observation of EVD can be useful for pregnancy prediction independent of the remaining examination results, if microbiological analysis is not feasible in the farm setting.

Key words: dairy cow, clinical endometritis, prediction, reproductive performance

Zusammenfassung

Vetsuisse-Fakultät Universität Zürich

Martina Ernstberger

Departement für Nutztiere
Ambulanz und Bestandesmedizin
cgeisinger@vetclinics.uzh.ch

Prognostizierbarkeit der Fertilität von Kühen mit klinischer Endometritis basierend auf einer Kombination von klinischen Symptomen und Untersuchungsergebnissen

Ziel dieser Studie war es, die Prognostizierbarkeit der Fruchtbarkeit von Kühen mit klinischer Endometritis unter Berücksichtigung verschiedener Parameter zu verbessern. Die Diagnose klinische Endometritis wurde bei 300 Kühen durch Beurteilung des Vaginalsekrets mit der Metricheck™-Methode bei der Puerperalkontrolle 1 (PK 1 = 22-45 Laktationstage) gestellt (Endometritis Score (ES) 1-3). Es wurde eine ausführliche Anamnese erhoben und allgemeine klinische und gynäkologische Untersuchungen sowie eine bakteriologische Beurteilung des Vaginalsekrets durchgeführt. Eine zweite Puerperalkontrolle erfolgte 21 Tage später. Puerperalerkrankungen vor Eintritt in die Studie, externer vaginaler Ausfluss (EVA) und der Nachweis von *Trueperella pyogenes* (TP) begünstigten die Entwicklung von ES 3. Der Nachweis von ES 3 bei der PK 1 war wiederum assoziiert mit reduzierter Fruchtbarkeit (längere Rastzeit, reduzierte Gesamtkonzeptionsrate, höhere Abgangsrate). Wurde die bakteriologische Untersuchung neben allen anderen Untersuchungsergebnissen in das multivariable statistische Modell mit einbezogen, war TP der entscheidende Faktor für die Prognose des Trächtigkeitsstatus am Tag 100, 150 oder 200 in Laktation; wurde dies nicht gemacht, war EVA der wichtigste Faktor. Somit eignet sich die Beobachtung von EVA für die Vorhersage der Fertilität, wenn eine mikrobiologische Untersuchung unter Feldbedingungen nicht durchführbar ist.

Stichwörter: Milchkuh, klinische Endometritis, Vorhersage, Reproduktionsleistung

Predicting fertility in cows with clinical endometritis based on a number of clinical symptoms and examination results

M. Ernstberger*[1], H. Oehl†, M. Haessig‡, S. Hartnack§ and H. Bollwein†

* Department of Ambulatory Service and Herd Health Medicine, and

† Department of Reproductive Medicine, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, Zurich, Switzerland

‡ AgroVet.Strickhof, Eschikon 27, Lindau, Switzerland

§ Section of Epidemiology, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, Zurich, Switzerland

[1]Corresponding author: mernstberger@vetclinics.uzh.ch

ABSTRACT

The objective of this study was to improve prediction of the reproductive performance in cows with clinical endometritis by means of a combination of examination results. For this purpose, a total of 1,386 cows from 33 herds were screened for clinical endometritis. Data from 200 cows without a corpus luteum (CL) and 100 cows with a CL were collected. The calving history, puerperal or concurrent diseases and cow-specific data (breed, parity, body condition score, and daily milk yield) were collected for each cow. The diagnosis of clinical endometritis at puerperal control 1 (PC 1 = 22-45 days in milk (DIM)) was made by vaginal discharge scoring with a Metricheck device on a scale from 1-3 (endometritis score (ES) 1-3). A detailed clinical and gynecological examination including external inspection, transrectal palpation and ultrasonographic examination of the genital tract (cervix, uterus, and ovaries), vaginoscopy and microbiological analysis of the intrauterine content were performed. All cows were treated according to the ovarian findings at PC 1. A second puerperal control was performed 21 days later. Reproductive data were recorded until 200 DIM. The risk factors for ES 3 were determined by the Chi-square test and binary logistic regression. The univariate effects of each risk factor on selected reproduction performance parameters were assessed. Multivariable implications of the risk factors on pregnancy status at different DIM times (100, 150 and 200 DIM) were analyzed on the basis of conditional inference trees. The prevalence of clinical endometritis was 28%. The ovarian findings at PC 1 did not have any impact on reproductive performance. Puerperal diseases prior to the study, external vaginal discharge (EVD) and the presence of *Trueperella pyogenes* (TP) favored the development of an ES 3. The presence of an ES 3 at PC 1 was associated with impaired fertility (higher number of days to first service, lower conception rate to all services, higher culling rate due to infertility). The prediction of pregnancy status for all DIM times was dominated by EVD or TP as decisive factors, depending on whether bacteriological findings were included in the model. Cows with EVD or TP had lower pregnancy rates at all DIM times than cows without these findings, but the predictive accuracy was similar for both groups of cows. These results suggest that EVD can be used for pregnancy prediction in cows with clinical endometritis if microbiological analysis is not feasible in the farm setting.

Key words: dairy cow, clinical endometritis, prediction, reproductive performance

INTRODUCTION

The primary goal in dairy cattle farming is economically efficient reproduction management to achieve farm-specific good herd reproduction parameters, such as an adequate time to pregnancy and a high conception rate. As the course of the puerperal period is essential for the subsequent reproductive performance, it is important to focus on early puerperal examinations in every cow (3-5 weeks after calving) to recognize animals with gynecological diseases and to intervene at an early stage (LeBlanc et al., 2002a, Sheldon et al., 2006). Clinical endometritis is one of the most common puerperal uterine diseases and has been defined by several authors over the last decade (LeBlanc et al., 2002a, Sheldon et al., 2006, Williams et al., 2005). Sheldon et al. (2006) defined clinical endometritis as purulent or mucopurulent vaginal discharge without any accompanying systemic signs beginning at 21 days after calving. The prevalence of this disease in dairy herds depends mainly on the time of examination during the puerperal period and varies from 14-42% (Drillich et al., 2005, Knutti et al., 2000, Pleticha et al., 2009, Westermann et al., 2010, Williams et al., 2005). Numerous studies have shown the effects of dystocia, twin birth, retained fetal membranes or metritis on the development of endometritis (Dubuc et al., 2010, Kim and Kang, 2003, LeBlanc et al., 2002a). The abovementioned risk factors lead to impaired function of neutrophil granulocytes, delayed uterine involution and damage to the uterus, which promotes nonpathogenic and pathogenic bacterial colonization (Foldi et al., 2006, Gilbert et al., 2005, Sheldon et al., 2006). The pathogenesis of endometritis confirms the importance of a consequent focus on the early puerperal period of a lactating cow. Diverse methods, such as uterine lavage, uterine cytology, endometrial biopsy or ultrasonographic measurements of the cervix and endometrial thickness, have been described as diagnostic tools for clinical endometritis (Barlund et al., 2008, Bonnett et al., 1993, Dubuc et al., 2010, LeBlanc et al., 2002a, Machado et al., 2012). In the field, the diagnosis of clinical endometritis is usually based on transrectal palpation of the uterus, which is quite subjective and has only low predictive value for the animal's reproductive performance (LeBlanc et al., 2002a). It is primarily complemented by ultrasonography, vaginal examination with a gloved hand, vaginoscopy, examination with a fairly new diagnostic tool, the Metricheck device (Simcro Tech, Hamilton, New Zealand), or a combination of these methods (McDougall et al., 2007, Pleticha et al., 2009, Senosy et al., 2009, Sheldon et al., 2002).

In addition to genetic factors and the animal's specific immune status, the severity of endometritis is mainly influenced by the presence of uterine pathogens, such as *Trueperella pyogenes* (**TP**), *Escherichia coli*, or *Fusobacterium necrophorum*, in the uterine lumen (Sheldon et al., 2006, Williams et al., 2005). *Trueperella pyogenes* is one of the most commonly

detected pathogens and often interacts synergistically with *Fusobacterium necrophorum* and other Gram-negative anaerobic bacteria, such as *Bacteroides spp.* and *Prevotella spp.* (Bekana et al., 1994, Ruder et al., 1981). Cows with intrauterine TP show impaired reproductive performance (Bonnett et al., 1993) including a prolonged time to pregnancy and a 34% lower likelihood of pregnancy than TP–negative cows (Bicalho et al., 2016).

In general, clinical endometritis has been demonstrated to cause a poor reproductive performance, such as a reduced pregnancy rate, an increased time to conception and culling rate, and a reduced first-service conception rate (**FSCR**) (Kim and Kang, 2003, LeBlanc et al., 2002a, Runciman et al., 2008, Sheldon et al., 2006). Furthermore, it has a negative impact on milk yield (Gilbert et al., 2005), which leads to considerable economic losses.

For dairy farmers and veterinary practitioners, it would be of great benefit to be able to predict the future reproductive performance of cows with endometritis early after parturition. In general, single risk factors for the pregnancy status of cows with clinical endometritis have been described, and only a few studies have estimated the reproductive performance of cows based on a number of diagnostic findings (Dubuc et al., 2012, Grohn and Rajala-Schultz, 2000, Kim and Kang, 2003, LeBlanc et al., 2002b, Williams et al., 2005). To date, studies have been conducted to predict the **FSCR** and the likelihood of pregnancy until 100, 200 and 250 DIM with different diagnostic tests such as vaginoscopy, endometrial cytology or leucocyte esterase tests (Barlund et al., 2008, Denis-Robichaud and Dubuc, 2015, Gobikrushanth et al., 2016) or to estimate the reproductive performance on the basis of clinical, bacteriological and histological findings until 175 DIM (Bicalho et al., 2016, Bonnett et al., 1993).

The main goal of this study was to investigate the possibility of predicting the reproductive performance of dairy cows with endometritis by using a number of clinical findings during the puerperal period for different DIM cut-offs.

MATERIALS AND METHODS

Farms and Animals

The study was conducted on 33 dairy farms managed by the Ambulatory Service of the Department for Farm Animals (Vetsuisse Faculty, University of Zurich, Switzerland) between October 2014 and July 2016 and was authorized by the Federal Veterinary Office of Switzerland (animal license numbers: eTV25249, ZH43/14). A total of 300 cows of four different breeds (35% Red Holstein, 29% Brown Swiss, 27% Holstein Friesian, and 9% Simmental) were included in the study. They were either housed in tie-stalls (n = 8) or in free-stall facilities with cubicles and slotted or concrete floors with rubber mats (n = 25). Two hundred cows in the study population were also examined for another study comparing the effects of different therapies of endometritis in cows bearing no corpus luteum (**CL**) on the ovaries at the first examination after parturition. For the present study, in addition, 100 cows with clinical endometritis and a CL > 20 mm in size were investigated. Cows meeting exclusion criteria defined before the start of the study were not enrolled in the examinations (culling due to farm-specific reasons, simultaneous antibiotic treatment due to infectious disease, or an inappropriate farm-specific waiting period). The herd sizes on the farms varied between 7 and 104 milking cows (39 ± 25 cows; mean ± SD). The study population was composed of 56 cows from farms with less than 30 cows and 244 cows from larger farms. The average 305-day herd milk production ranged between 3,700 and 14,100 kg (8,095 ± 1,876 kg) at a lactation number of 3.3 ± 2.1 (range: 1 - 9 lactations). In most of the two husbandry systems, the cows were offered regular grazing times or access to free-range areas throughout the whole year. Feeding was arranged individually by farm and mainly consisted of a TMR based on grass silage, maize silage, hay and supplementation with minerals and concentrates adjusted to the cow's individual current milk yield (average daily milk yield: 33 ± 8 kg per cow, range: 17 – 60 kg/d per cow). In 31 farms, the cows were milked by a manually handled milking system twice a day. Two of the involved farms were equipped with an automatic milking system that the cows attended approximately 2.6 times per day. Calvings were evenly distributed throughout the year. The voluntary waiting period was handled individually by the farmers and varied considerably (35 – 90 days). The cows were mainly examined by two of the authors (M. I. E. and H. O.) with a few exceptions.

Study Design

In all lactating cows from the participating farms, diagnostic screening for endometritis was performed between 22 and 45 DIM. When clinical endometritis was evident, additional

information about the cow and its periparturient period was obtained by a questionnaire followed by a detailed gynecological and short clinical examination. This first examination of the study protocol was defined as puerperal control 1 (**PC 1**; Figure 1). Additionally, uterine cytobrush samples were taken to analyze the bacterial colonization of the uterine contents. After the sampling procedure, the cows were treated according to their ovarian findings. Three weeks (21 ± 1 d) after the first examination (43 - 66 DIM), each cow underwent a second puerperal control (**PC 2**) and again received a subsequent hormonal treatment adjusted to the ovarian findings. Cows without any signs of clinical endometritis where then inseminated artificially at the next apparent estrus or later in farms with a prolonged voluntary waiting period. In contrast, if they still showed an endometritis score (**ES**) 1-3, the cows were evaluated and re-examined every 14 - 21 d and treated hormonally until an ES 0 was attained, and at that time the cows were ready for artificial insemination (**AI**). Finally, 30 - 37 d after AI, an ultrasonographic pregnancy check was performed. To rule out the occurrence of embryonic death, a second pregnancy check was performed 50 - 64 days after AI. Nonpregnant cows were evaluated at the next heat for a subsequent AI procedure. In cases of an absent heat, the cows were re-examined every two weeks and treated hormonally according to the ovarian findings until successful insemination was achieved.

The cows were followed until 200 DIM. Cows that did not become pregnant from insemination by 200 DIM at the latest were culled due to infertility, and additional data were not collected.

Animal Selection and Endometritis Scoring

To diagnose cows with endometritis, visual inspection of the cows' perineal area including the tail and a vaginal examination with a Metricheck device (Simcro Tech, Hamilton, New Zealand) to evaluate for vaginal discharge were performed. To prevent the genital tract from further contamination, the vagina and the perineal area were cleaned with water and a disinfectant soap based on a 0.75% povidone-iodine solution (Betadine liquid soap, Mundipharma Medical Company, Basel, Switzerland). After the cleaning process, the Metricheck device was lubricated with sterile saline solution and inserted into the vagina just near the external os of the cervix. Vaginal discharge was collected in the concave rubber cup of the Metricheck by moving the device along the dorsal, lateral and ventral wall of the vaginal cavity and holding the stick slightly upwards while pulling it caudally back out of the vagina. After each usage, the Metricheck device was disinfected with a 10% povidone-iodine solution (Betadine solution, Mundipharma Medical Company, Basel, Switzerland). Vaginal discharge was evaluated on the basis of the widely accepted scoring system developed by Sheldon et al. (2006) on a scale from 0 to 3. Cows with a clear or translucent mucus (score of 0) were

considered clinically healthy cows. An ES of 1 (**ES 1**) referred to mucus with flecks of white or off-white pus, and a score of 2 (**ES 2**) was classified as vaginal discharge containing less than 50% white or off-white mucopurulent material. A score of 3 (**ES 3**) was equivalent to mucus with more than 50% purulent discharge, which was mostly yellow or white and in some cases sanguineous. To evaluate the origin of the vaginal discharge and thus rule out the occurrence of a vaginitis or cervicitis, the origin of the discharge from the uterus was confirmed by vaginoscopy.

Data Collection at Puerperal Control 1

The anamnesis included animal-specific information (breed, parity (primiparous, multiparous), BCS on a scale from 1 - 5 with half scores (Ferguson et al., 1994), season of calving, current daily milk yield, puerperal diseases after parturition (retained placenta, metritis), previous or current presence of external vaginal discharge (**EVD**), concurrent diseases at the time of the study enrollment) and farm-specific questions (housing, farm and herd size, milking procedure). Three of the smaller farms were not registered in the national herd book; therefore, no results regarding a milk analysis existed, so the daily milk yield of the cows from these farms was estimated by the farmers.

Initially, each cow underwent a transrectal manual assessment of the uterus size and possible uterine fluctuation. By the use of a portable ultrasound device (ProXima Pavo Pro, Proxima Medical Systems AG, Pratteln, Switzerland, linear probe, 7.5 MHz) cross-sectional images were obtained of the cervix, the uterine body, the uterine horns (~2 cm caudally from the uterine body bifurcation, Souza et al. (2011)) and the tips of the uterine horns and saved for subsequent evaluation of the visible uterine content and the cervical diameter of each cow. Likewise, ultrasonographic images of the ovaries were obtained and saved for further evaluation. To assess the cervical diameter, the computer-assisted image analysis program PixelFlux was used (Version 1.0; Chameleon Software, Leipzig, Germany). The measurements were repeated twice and performed by the main two authors who were blinded to the other examination results of each cow. The cervical diameter was calculated by the mean of two cross-sectional diameter values. Uterine content was documented where an echogenic filling of the uterus was seen in at least one of the uterine images.

Furthermore, a double-guarded cytobrush (Cytology Brush, Minitube GmbH, Tiefenbach, Germany) sample for bacteriological examination of the uterine content was taken. After cleaning the vagina with povidone-iodine again, the device, which was protected with a synthetic cover, was inserted with a gloved hand through the parted vaginal lips into the vaginal vestibule before it was guided manually into the external cervical os under rectal manual

guidance. Once the tip of the cytobrush stick was located at the external os of the cervix, the plastic cover was pierced by the flexible catheter located inside. The brush at the tip of the device inside the flexible catheter was passed through the cervix and finally placed in the uterine lumen. Afterwards, it was gently rubbed along the uterine endometrium and pulled back by covering the brush-end again. Finally, the brush was wiped off on a sterile swab (Transystem, COPAN, Brescia, Italy) and stored refrigerated for a later bacteriological analysis. The microbiological analysis of the swabs was performed by the Institute for Veterinary Bacteriology, University of Zurich, Switzerland, within a maximum of 12 h after sampling. The swabs were streaked on different plates for culturing aerobic and anaerobic bacteria and incubated for 48 h at 37°C before further analysis and identification of subcultures was performed.

Treatment Schedule

At PC 1, the 200 cows not bearing a CL on the ovaries were randomly allocated into two treatment subgroups of 100 animals each. In 100 cows, 0.1 mg gonadorelin (GnRH, Fertagyl, MSD Animal Health GmbH, Luzern, Switzerland) was injected intramuscularly (i.m.). If no follicles ≥ 9 mm were present on the ovaries, the cows received in addition an intravaginal progesterone-releasing device (1.9 g progesterone, EAZI-BREED CIDR, Pfizer AG, Zurich, Switzerland). Seven days after the placement of the intravaginal device (29 - 52 DIM), it was removed by the farmer and a simultaneous i.m. injection of 0.5 mg cloprostenol ($PGF_{2\alpha}$ derivate; Estrumate, MSD Animal Health GmbH, Luzern, Switzerland) was administered. The remaining 100 cows without a functional CL were treated with an intrauterine infusion of 500 mg cefapirin (Metricure, MSD Animal Health GmbH, Luzern, Switzerland). The antibiotic suspension was dispensed into the uterine lumen with a single use flexible catheter (Metricure, MSD Animal Health GmbH, Luzern, Switzerland). The 100 cows with a functional CL at PC 1 received a single i.m. injection of 0.5 mg cloprostenol to stimulate luteolysis and induce estrus.

At PC 2, the cows were checked again by using Metricheck and transrectal ultrasonography. The ES, odor of the discharge and ovarian findings of each cow were noted. All cows with a CL received an i.m. injection of 0.5 mg cloprostenol. In cases of cystic ovarian follicles or missing functional bodies on the ovaries, the cows were either treated according to the previous described protocol with 0.5 mg GnRH i.m. or a progesterone-releasing intravaginal device followed by an injection of 0.5 mg cloprostenol ten days later, after consultation with the farmer.

Data Management and Statistical Analysis

All the data from the cows were recorded in a Microsoft Excel file (Microsoft Corporation, One Microsoft Way, Redmond, WA, USA). Statistical analyses were performed with IBM SPSS (IBM SPSS Statistics Version 22, IBM Switzerland Ltd, Zurich, Switzerland) and the statistical software R (R Foundation for Statistical Computing, Vienna, Austria).

The risk factors were recorded nominally or ordinally. Body condition scores for Red Holstein, Holstein Friesian and Brown Swiss cows during early lactation were categorized as under-conditioned (< 2.5), adequately conditioned (2.5 - 3.25) and over-conditioned (> 3.25). For Simmental cows, the category limits were increased by a half point (Heuwieser and Mansfeld, 1992). The season of calving was summarized as follows: spring from March-May, summer from June-August, autumn from September-November and winter from December-February. The individual daily milk yield of the cows was classified in three groups: 15 – 30 kg, 31 – 45 kg and 46 – 60 kg. The uterine size was defined as follows: size 1 = uterus delimitable by hand, size 2 = uterus nearly delimitable, size 3 = uterus undelimitable by hand and mainly situated outside the pelvic cavity, and the cervical diameter was classified as < 5 cm (3.35 – 4.99) or ≥ 5 cm (5.00 – 7.75) (Sheldon et al., 2006).

To evaluate the effect of individual risk factors (data from the anamnesis and the examination at PC 1) on the distribution of ES at PC 1, a Chi-square analysis was performed. In cases of n < 5, Fisher's exact test was chosen. Univariate associations between the risk factors with p-values < 0.05 in the Chi-square analysis and the development of an ES 3 were tested with a binary logistic regression approach. Correlations of the intrauterine bacteria were analyzed using Spearman's rank correlation.

A descriptive and univariate statistical analysis of the effects of each variable on selected fertility parameters (**DFS**: days to first service, **DO**: days open, **CRI**: culling rate due to infertility, FSCR, **CRAS**: conception rate to all services, **TCR**: total conception rate, definitions listed in Table 1) was performed. As there were no significant differences between the various treatment protocols on fertility parameters (Kruskal-Wallis ANOVA and Chi-square test), all cows were regarded as one population for further calculations, regardless of their initial treatment. The univariate effect of the contributing factors on the reproductive performance considering the data until 200 DIM was evaluated by using the fertility parameters mentioned above. Regarding the measurements of the cervical diameter, the interobserver agreement was moderate (Cohens kappa test of agreement: $\kappa = 0.48$), which confirmed the reproducibility of the measurements.

As the numeric reproduction performance parameters were not normally distributed (in accordance with Kolmogorov-Smirnov test), the median DFS and DO of each binomial predictor variable were compared with the nonparametric Mann-Whitney *U*-test. If a parameter with more than two subsets was analyzed, the nonparametric Kruskal-Wallis ANOVA was performed. In cases of a significant outcome, a post hoc Dunn-Bonferroni test was performed to assess differences between the single subsets. The parameters DFS and DO are presented in the tables as the mean ± SD. A univariate analysis of the effects of the study parameters on FSCR, CRAS and CRI was performed using the Chi-square test.

In the second step, a multivariable analysis of effects of the risk factors on the pregnancy status at different DIM times (100 DIM = $\mathbf{DIM_{100}}$, 150 DIM = $\mathbf{DIM_{150}}$, 200 DIM = $\mathbf{DIM_{200}}$) was performed. With the aim of constructing a predictive model, a recursive partitioning approach including conditional inference trees and random forests was performed. The idea was to partition the covariate space and compute single statistics of the dependent variables inside each cell (Hothorn and Zeileis, 2015). The advantage of this approach is that typically more predictors including interactions compared to a classical regression approach can be handled. Conditional inference trees were obtained with the function “ctree” of the R package partykit (Hothorn et al., 2006, Hothorn and Zeileis, 2015), and random forests with the same predictors (package random forest (Liaw and Wiener, 2002)) were used to assess the sensitivity and specificity.

The level of significance was set at $P < 0.05$, and values < 0.01 were treated as highly significant. The values between 0.05 and 0.10 were regarded as tendencies.

RESULTS

In total, 1,386 cows were screened for signs of endometritis. The prevalence of clinical endometritis was 28% (n = 385). In 34% (n = 131) of the cows with endometritis, at least one CL with a diameter ≥ 20 mm was detected. In the study protocol, 300 lactating cows (without CL: n = 200, with CL: n = 100) with clinical endometritis at a median study entry of 28 DIM (minimum: 21 DIM, maximum: 45 DIM) were included. Eighty-five cows diagnosed with clinical endometritis were not enrolled due to the exclusion criteria mentioned before. An overview of the numbers and proportions of the cow-specific data and examination results is provided in Tables 4-6.

Endometritis Score

In total, at PC 1, ES 1 (n = 105, 35%) and ES 2 (n = 120, 40%) were diagnosed more frequently than ES 3 (n = 75, 25%) ($P < 0.01$). Puerperal diseases and EVD showed a significant influence on the occurrence and the degree of the ES (Table 2). Both anamnestic factors were associated with an increased risk of ES 3 at PC 1 (Table 3). In a total of 300 cows with a vaginal discharge as detected by Metricheck, 104 cows (35%) also showed vaginal discharge around the perineum.

Cows with a CL more frequently ($P = 0.01$) had an ES 3 than cows without a CL. The prevalences of ES 1 and ES 2 were independent of the presence of a CL on the ovaries. In cows without a CL an ES 3 was less common ($P < 0.01$) than an ES 1 or ES 2, while in cows with a CL the ES were equally distributed. Cows with higher ES showed more frequently a fetid odor ($P < 0.01$). In 61% of the cows with an ES 3, a fetid odor of the vaginal discharge was found, compared to 5% of the cows with an ES 1 ($P < 0.01$). In only 13% of the cows with neutral smelling discharge, an ES 3 was detected. The risk of finding an ES 3 at the gynecological examination was 10.7 times higher ($P < 0.01$) for cows with a fetid odor of the vaginal discharge compared to cows with a neutral smelling vaginal discharge. If uterine fluctuation was palpable, ES 3 was more frequently diagnosed than in cows without uterine fluctuation ($P = 0.05$). In 16% of the cows with uterine fluctuation, an ES 1 was diagnosed compared to 46% and 38% of cows with an ES 2 or 3, respectively.

Diagnosis of an ES 3 was associated with the presence of TP in the uterus in 50% of the cows. In comparison, only 18% of the cows with an ES 1 were affected by TP ($P < 0.01$). The risk of cows having an ES 3 was 5.87 times higher in cows with TP than in cows without TP.

The season of calving (number of calvings in spring: 42 (14%), summer: 86 (29%), autumn: 115 (38%), winter: 86 (29%)), parity, BCS, concurrent diseases, milk yield and bacteria other

than TP had no significant effect on the degree of endometritis. In addition, the sonographic appearance of the uterine content was not associated with the ES. However, fewer animals ($P < 0.01$) with a cervical diameter < 5 cm showed an ES 3 than animals with a larger uterine cervix. The odds ratio (**OR**) for cows with a cervical diameter ≥ 5 cm to have an ES 3 was 2.15. At PC 2, data from 298 cows were available. Two cows were culled prior to PC 2 due to claw disorders. In 54% (n = 160) of the cows, ES 0 was registered, and ES 1, ES 2 and ES 3 were diagnosed in 30% (n = 91), 9% (n = 26) and 7% (n = 21) of the cases, respectively. In 217 cows (73%), an improvement in the vaginal discharge to a lower ES from PC 1 to 2 was attained. In 62 cows (21%), the ES was consistent, and 38 cows (13 %) were diagnosed with worsening vaginal discharge. The percentage of cows with a fetid vaginal discharge decreased ($P < 0.01$) from 22% to 7% between PC 1 and PC 2. Similar to PC 1, the percentage of cows with a fetid odor of the vaginal discharge was higher in cows with ES 3 than in cows with lower ES ($P < 0.01$). There was no association between the presence of a CL at PC 2 and the type of discharge.

Bacteria

Bacteriological samples were obtained and cultivated as described above from 296 cows. Four samples could not be analyzed due to fecal contamination (n = 2) or difficulties with the sampling technique (n = 2). In seven samples, no bacteria were detected, whereas up to six different genera per culture were found in the remaining 289 samples. The eight most frequently identified genera are shown in Figure 2. The genera, which occurred with a frequency of less than 0.5% (n = 18), were summarized to "*Others*" (*Coagulase positive Staphylococci,* Gram-positive Anaerobes, *Bacillus licheniformis, Pseudomonas aeruginosa, Acinetobacter spp., Proteus spp., Pantoea spp., Mannheimia haemolytica, Enterobacter spp., Clostridium perfringens, Corynebacterium bovis, Pasteurella multocida, Micrococcus spp., Histiophilus somni, Actinobacillus spp., Lactococcus garvieae* and *Bibersteinia trehalosi*).

In most of the bacteriological cultures, three different genera were identified (n = 78, 26%). In 75 samples (25%), two genera were detected followed by pure cultures (n = 59, 20%) and cultures with four (n = 56, 19%), five (n = 17, 6%), or six different genera (n = 4, 1%). Overall, *E. coli* (n = 102) and TP (n = 90) were the most predominant genera followed by *Serratia spp.* (n = 85). The pure culture samples were dominated by *E. coli* (n = 19, 32%) and *Serratia spp.* (n = 16, 27%). *Trueperella pyogenes* was mostly accompanied by Gram-negative anaerobes (47%); this was the only species in which a positive correlation with the occurrence of TP could be proven ($r_s = 0.47$, $P < 0.01$). *E. coli* was the second most common microbe found in TP-positive swabs (34%) followed by *Serratia spp.* (24%), but no significant correlation was

evident. Instead, samples with *E. coli* showed a negative correlation with the third most common finding of *Serratia spp.* (r_s = -0.37, $P < 0.01$) and *CNS* (r_s = -0.22, $P < 0.01$).

Univariate Effects of Anamnestic, Clinical and Bacteriological Findings on Reproductive Performance

The mean DFS was 80 ± 27 days, and the mean number of days from calving until successful insemination (DO) for cows that became pregnant within 200 DIM (n = 206) was 106 ± 41 days. The FSCR of the cows showing clinical endometritis was 33% (n = 100). Within 200 DIM, 69% (n = 206) of cows with clinical endometritis became pregnant (TCR) at a CRAS of 34% (206/600, Table 1).

Furthermore, 71 cows (24%) were culled due to infertility or were not further pursued after 200 DIM, and 23 (8%) cows were culled because of miscellaneous reasons (e.g., udder health, claw disorders). Overall, 19 cows (6%) were culled within the voluntary waiting period of their respective farms due to milk yield or claw disease issues and were therefore not inseminated.

Anamnestic findings. There was no significant difference in the fertility parameters between primi- and multiparous cows (Table 4). Over-conditioned cows had a shorter period of DO than adequately conditioned cows ($P = 0.02$). In contrast, the DFS was not affected by body condition. Over-conditioned cows had the best FSCR and CRI results compared to adequately or under-conditioned cows ($P = 0.02$).

Puerperal disease prior to the study led to a significant extension of the period of DO in cows with these diseases compared to cows without such diseases. Likewise, FSCR and CRAS were higher in unaffected cows than in cows that had a puerperal disease ($P = 0.02$ and $P = 0.01$). In the latter group, the proportion of cows culled due to infertility tended ($P = 0.05$) to be higher than in healthy cows.

The average DO was numerically higher in cows with EVD, but DO and DFS did not differ significantly between cows with and without vaginal discharge observed by the farmer or examiner. The presence of EVD within the period between calving and enrollment in the study was indicative of profound negative effects ($P < 0.01$) on the FSCR, which was almost twice as high in cows without obvious vaginal discharge compared to cows with EVD. Accordingly, the CRAS was significantly lower, and the percentage of cows culled due to infertility was higher ($P < 0.01$) in cows with vaginal discharge after calving than in those without such discharge.

Regarding the daily milk yield of the cows at PC 1, the median DFS and DO were lower in cows with lower milk yield (15 - 30 kg/d) than in those with higher milk yield. In cows with

the highest milk yield of the study population (> 45 kg/d), the FSCR was distinctly reduced (14% *vs*. 40% in cows with ≤ 30 kg/d, $P < 0.01$), and the CRAS was only 25% compared to cows with the lowest milk yield (38%, $P = 0.05$).

Clinical Findings at Puerperal Control 1. The type of treatment and the presence of a CL on the ovaries at PC 1 had no significant effect on any of the evaluated fertility parameters (Table 5). Cows with a uterine size of 2 had the numerically highest FSCR (41%), but a significant difference was only found compared to a uterine size of 3. Additionally, the CRAS was remarkably higher and the risk for culling due to infertility was lower in cows with a uterine size of 1 or 2 ($P = 0.02$ and $P < 0.01$) than in cows with a uterine size of 3.

Cows showing an ES 3 at PC 1 had a longer period from calving to first insemination than those with an ES 2 ($P = 0.02$), while no significant difference was observed in this fertility parameter between cows with ES 1 and ES 3. The mean DO was slightly longer in cows with an ES 3 than in those with ES 1 or 2, but it was not significantly affected by the ES at PC 1. Cows with an ES 3 had a lower CRAS and a higher risk of being culled due to infertility than cows with an ES 2 ($P = 0.04$).

In cows with a fetid vaginal discharge, the period from calving to first AI tended ($P = 0.06$) to be longer than in cows with odorless discharge, but the nature of the odor was of minor relevance in terms of the fertility of the cows (all fertility parameters: $p > 0.05$).

The cows with palpable uterine fluctuation had shorter DFS ($P < 0.01$) than those without palpable uterine fluctuation, but the sonographically assessed uterine findings of the visible uterine content and cervical diameter (minimum: 3.35 cm, maximum: 7.75 cm) did not have an impact on the fertility parameters at all.

As TP was the only intrauterine bacteria that had significant negative effects on all the investigated fertility parameters, bacteria other than TP were not further evaluated or reported in the tables. In cows with TP, the DFS, DO and CRI were higher and the FSCR and the CRAS were lower than in cows without TP in the uterine lumen.

Clinical Findings at Puerperal Control 2. Cows with an ES 0 or ES 1 at PC 2 were inseminated significantly earlier after parturition than cows with ES 2 or ES 3. In contrast to the DFS, the DO did not differ based on the ES at PC 2. However, only 6 out of 21 (29%) cows with an ES 3 at PC 2 became pregnant within 200 DIM. Similar impaired effects of an ES 3 at PC 2 were found for the FSCR and the CRAS. In cows with ES 3, the FSCR was lower ($P = 0.03$) than in cows with ES 0 (FSCR: 13% *vs.* 40%). Cows with ES 3 also had a higher risk ($P < 0.01$) of being culled due to infertility than cows with lower discharge scores. If a fetid odor

of the vaginal discharge at PC 2 was recorded, the cows tended to have longer DFS ($P = 0.06$) and DO ($P = 0.07$) than cows with a neutral smelling vaginal discharge.

Cows with inactive ovaries and those with a CL had notably higher FSCR values than cows with dominant follicles at that time ($P = 0.03$). In cows with dominant follicles, CRAS values were lower (27%) than in cows with a functional CL (37%, $P = 0.03$).

Multivariable Analysis of Predictability of the Pregnancy Outcome

The total conception rates (Table 1) at $DIM_{100,}$ DIM_{150} and DIM_{200} were 46% (106/229), 63% (173/275) and 74% (206/279), respectively.

When the anamnestic data and examination results (except ultrasonography) of PC 1 were taken into account (Table 7), the presence of EVD was definitive for predicting the pregnancy rate for the considered DIM cut-offs ($P < 0.01$). Of all cows with EVD, 22%, 42% and 58% became pregnant until DIM_{100}, DIM_{150} and DIM_{200}, respectively. When no vaginal discharge was visible, 65% and 76% of the cows became pregnant until DIM_{150} and DIM_{200}, respectively. For prediction of the likelihood of pregnancy at DIM_{100}, additionally, the individual daily milk yield was importantin cows without EVD ($P < 0.01$). Of the cows without EVD and a milk yield of $\leq$ 43 kg/d, 47% became pregnant until DIM_{100} compared to cows with a higher milk yield (11%). There was no difference in the outcomes when ultrasonographically determined parameters (any uterine content, cervical diameter) of PC1 were added to the statistical model. The exact pregnancy rates for each model are given in Table 7.

When considering the results of PC 1 and the microbiological analysis, TP was the main decisive risk factor for the pregnancy outcomes at all DIM cut-offs. If TP was present in the uterus at PC 1 (n = 90), 20% of the cows became pregnant at DIM_{100} and 38% and 54% became pregnant at DIM_{150} and DIM_{200}, respectively. Cows without TP in the uterine lumen had significantly higher pregnancy rates (Table 7). For DIM_{100} as the outcome variable, the daily milk yield was the most decisive factor in cows without TP. The decision threshold of the daily milk yield (determined by the statistical program) was 34 kg/d compared to 43 kg/d for the model with only the data of PC 1. Cows without TP and a daily milk yield of less than 34 kg/d had a higher chance ($P < 0.01$) to become pregnant until 100 DIM than cows with a higher milk yield (51% vs. 24%).

When considering the available examination data from PC 1 and PC 2 together, EVD was an outstanding predictive factor for all considered DIM cut-offs ($P < 0.01$). For DIM_{100}, the outcome remained the same compared to the model based only on information from PC 1. For DIM_{150} and DIM_{200}, the pregnancy outcomes of cows without vaginal discharge prior to PC1

depended on the ES at PC 2 ($P < 0.01$). Thus, ES 3 at PC 2 in combination with absent EVD prior to the study was associated with low conception rates at DIM_{150} and DIM_{200} ($P < 0.01$). Finally, when all available data were used in the statistical model, TP was shown to have the greatest effect as a predictive factor for all considered DIM cut-offs. Nevertheless, for DIM_{100}, milk yield was the second decisive factor, and in addition, the ES on PC 2 was a crucial factor for cows with no TP in the uterine lumen but with a milk yield higher than 34 kg/d.

Altogether, the sensitivity for all models to predict pregnancy until 200 DIM was 90%, with a 20% specificity. The sensitivity for pregnancy prediction for DIM_{150} was lower (70%). The nonpregnancy rate for DIM_{150} could be predicted with a likelihood of 40%. For the period of DIM_{100}, the pregnancy rate was predictable with a probability of 25%. In addition, for this cut-off, the specificity was 85% (Table 7).

DISCUSSION

The prevalence of endometritis was below the range reported by earlier trials using rectal palpation (Drillich et al., 2005) or vaginoscopy (Westermann et al., 2010) solely, or a combination of clinical examination and endometrial cytology (Gobikrushanth et al., 2016) as diagnostic methods. It was even lower than in studies using the Metricheck technique in comparison with other diagnostic tools (McDougall et al., 2007, Pleticha et al., 2009) but higher than with manual vaginal examination (Williams et al., 2005) and equivalent to the study of Dubuc et al. (2010) (cytology and Metricheck together). Due to different diagnostic criteria, management factors and definitions of clinical endometritis or divergent postpartum examination periods, it is difficult to compare the results among studies. Similar to the trial by Knutti et al., 2000, in the present case, a prolonged screening period (until 45 DIM) was chosen compared to the studies mentioned above. A diagnosis of clinical endometritis in the late postpartum period leads to a higher chance of a preceding estrus due to the return of ovarian cyclic activity contributing to uterine emptying and thus self-resolution (LeBlanc et al., 2002a, Sheldon, 2004). In accordance with earlier findings, this leads to a lower prevalence of clinical endometritis (Gautam et al., 2009, Senosy et al., 2009, Williams et al., 2005). But as the median study entry was in the first third of the examination period and two thirds of the study population did not bear a CL at the ovaries, it can be assumed that the self-resolution effect was quite low.
The prevalence of clinical endometritis in primi- and multiparous cows is described controversely. Runciman et al. (2008) diagnosed more primiparous than multiparous cows with clinical endometritis, whereas Hendricks et al. (2006) did not report any age predisposition in cows with a history of a periparturient disease and a repeated treatment with $PGF_{2\alpha}$ before enrollment in the study. In this study, more multiparous than primiparous cows were diagnosed with clinical endometritis, but a statement about the effect of parity on the prevalence of clinical endometritis cannot be made as the data does not allow an assessment of the absolute numbers of primi- and multiparous cows of each participating farm.
Clinical endometritis was diagnosed with the Metricheck device, given its good on-farm suitability and because it is fast and easy to perform without any negative effects on the cow's subsequent reproductive performance (Pleticha et al., 2009). In another work, the terms "gross vaginal inflammation" (McDougall et al., 2011) or "vaginal mucus" (Senosy et al., 2009) were preferred since only purulent material in the vagina was detected by the Metricheck or vaginoscopy techniques. For this reason, in our study, the Metricheck findings were confirmed by vaginoscopy and ultrasonography to reduce the risk of false positive findings of clinical

endometritis. Cows without vaginal discharge determined by the Metricheck device were not further examined, which may have led to false negative results and a reduced prevalence of endometritis, as inflammation of the uterus may still have existed in these cows (Barlund et al., 2008, Sheldon et al., 2006). However, the sole use of Metricheck for diagnosis of endometritis has been proven to have a higher sensitivity than that of vaginoscopy (McDougall et al., 2007, Senosy et al., 2009). The relative risk of diagnosing ES with the Metricheck device was determined to be 1.29 times higher than with vaginoscopy (Pleticha et al., 2009), and others only found moderate agreement between these two methods (Runciman et al., 2009). Comparing uterine cytology and Metricheck scoring on a 0-5 point scale, the agreement was quite low (McDougall et al., 2011), but a significant correlation of the proportion of PMN and mucopurulent or purulent discharge have been proven (Barlund et al., 2008, Westermann et al., 2010). Dubuc et al. (2010) and Gobikrushanth et al. (2016) investigated a novel classification of postpartum uterine diseases as clinical (purulent vaginal discharge and/or intrauterine fluid), cytological (≥ 6 and 8 % PMN) or clinical and cytological endometritis. Both reported a significantly reduced risk of pregnancy until 250 DIM in cows with clinical and cytological endometritis. This novel diagnostic scheme seems to be most effective, but uterine cytology was not performed in the present study, as it is not practicable in farm conditions.

Since previous studies demonstrated a negative impact of ES 2 and ES 3 on fertility (Kaufmann et al., 2010, McDougall et al., 2007, Pleticha et al., 2009), the present study focused *inter alia* on associations between anamnestic data and examination findings, leading to the diagnosis of ES 2 and 3, respectively. Furthermore, risk factors for the development of an ES 3 were identified, as ES 3 turned out to have the strongest impact on the reproductive performance parameters in this study. Cows with an ES 3 at PC 1 had a significantly higher median of DFS, a lower CRAS and an increased CRI than cows with lower ES, which confirms previous findings (McDougall et al., 2007). Cows that still suffered from an ES 3 at PC 2 were found to have an even lower CRAS and a remarkably higher CRI than cows with lower ES. Furthermore, an ES 3 at PC 2 in combination with the absence of EVD before the study entry was predictive of a low chance of pregnancy at DIM_{150} and DIM_{200}.

In the univariate analysis, we confirmed that reproductive performance depends on the occurrence of puerperal diseases prior to the study (McDougall, 2001), visible vaginal discharge around the perineum (LeBlanc et al., 2002a), fetid odor of the vaginal discharge (LeBlanc et al., 2002a, Williams et al., 2005) and intrauterine growth of TP (Williams et al., 2005). The presence of these risk factors was associated with higher ES, and TP and a fetid odor of the

vaginal discharge were shown to be the most important risk factors for the development of an ES 3 (Williams et al., 2005).

Uterine involution after parturition takes approximately 40-50 days under physiological circumstances, and a cervical diameter < 5 cm at 40 DIM is an indicator of a successful puerperium (Gier and Marion, 1968, Sheldon et al., 2006). In addition to puerperal diseases, insufficient uterine involution can be related to other factors, such as age, breed or nutrition status (Fonseca et al., 1983), which differed widely between the participating herds in this study. In contrast to LeBlanc et al. (2002a) and Dubuc et al. (2010), who categorized the cervical diameter in different groups (< 5 cm, 5 – 7.5 cm and > 7.5 cm), in our study, the cows were only divided into two groups according to the cervical diameter (< 5 cm and ≥ 5 cm), because only one cow out of 300 (0.3 %) had a cervical diameter of > 7.5 cm. It remains unclear why we did not find more cows with a cervical diameter of > 7.5 cm, as the measurement procedure described by LeBlanc et al., 2002a was not described in detail, and the puerperal examination period was quite similar to ours (20 – 33 DIM vs. 22 – 45 DIM), but this finding was likely due to estimation of the cervical diameter based on transrectal palpation, which is less precise than the image-based measurements used in the present study. A larger cervical diameter was associated with a higher risk of developing an ES 3, but in contrast to the findings of LeBlanc et al. (2002a), no effects on the fertility could be proven; therefore, in our study, it was not a suitable parameter to predict fertility of the respective cows. In accordance with other studies, the clinical findings obtained by manual transrectal palpation (uterine size and fluctuation of the uterine contents) provided negligible information for the prediction of fertility compared to those derived from the character of the discharge (LeBlanc et al., 2002a, Runciman et al., 2008). The time of resumption of ovarian cyclicity was significantly associated with the severity of clinical endometritis but not with pregnancy outcomes. Cows with CL at PC 1 more often showed an ES 3. The odds of finding an ES 3 with the Metricheck device in these cows was twice as high as in cows without cyclic activity. Senosy et al. (2009) also found a higher Metricheck score in cows bearing a CL at 6 weeks postpartum compared to those cows without a CL. The specific time points of the previous estrus of cows with a CL in the present study were not recorded, but presumably they had ovulated shortly before, which led to uterine clearance by estradiol secretion and subsequent expulsion of purulent discharge into the vaginal cavity. In cows without a CL, intrauterine fluid was more frequently diagnosed by ultrasound, which was also a sign of missing uterine emptying (Senosy et al., 2009). Ultrasonographic detection of intrauterine fluid at 15 – 21 DIM was associated with a reduced FSCR at 70 DIM (Lopez-Helguera et al., 2012). We did not investigate the correlation between Metricheck

findings and the uterine contents in terms of the presence of a CL, as neither ovarian cyclicity nor ultrasonographically visible intrauterine fluid detected at 21 - 45 DIM showed any effect on the fertility of the study population. The possible confounding effects of the treatments at PC 1 and the following hormonal treatments to maintain the ovarian cyclicity in the present study were not further investigated as the treatment and the ovarian status of the cows also had no effect on the subsequent fertility.

Concerning the predictability of the reproductive performance of cows suffering from clinical endometritis, EVD was the best decision factor in both the univariate and the multivariable analyses and was associated with distinctively impaired fertility. Without a significant effect on DFS and DO, cows with vaginal discharge around the perineum had a poorer FSCR, CRAS and CRI than cows without vaginal discharge. When the different combinations of examination results were considered, EVD was the leading predictor followed by the current daily milk yield (for predicting pregnancy at DIM_{100}) or by the ES at PC 2 (for DIM_{150} and DIM_{200}) if no microbiological analysis was considered in the statistical model. LeBlanc et al. (2002a) reported a lower percentage of cows with visible perineal vaginal discharge than in our study. However, likewise, in a statistical model considering a history of reproductive disorders, the results of manual rectal palpation and the presence of vaginal discharge (around the perineum and/or on vaginoscopy) indicated that vaginal discharge had a negative impact on the likelihood of pregnancy in addition to the cervical diameter. McDougall (2001) reported that in only one cow out of 19 with clinical endometritis, vaginal discharge was also detected by the farmer before the study. Conversely, in four cows, vaginal discharge was seen before study entry, but this finding could not be confirmed by vaginoscopy. Therefore, in our study, besides the anamnesis given by the farmer, inspection of the perineum and the Metricheck results were combined to reduce the risk of a false positive diagnosis. Only in a few cases could no EVD be seen at PC 1 by the veterinarian, in contrast to the observations of the farmer. If a diagnosis of clinical endometritis was made in these cows, the presence of EVD was recorded for further analyses, anyway, as permanent efflux of the purulent material could not be expected due to ovarian cyclicity.

A higher ES at PC 1 was associated with negative effects on DFS, CRAS and CRI; however, in comparison with the observations of EVD, it was of minor importance for predicting pregnancy outcomes. This may suggest that EVD reflects the intrauterine conditions and is therefore a valuable indicator for the severity of clinical endometritis. A possible correlation between the uterine size and EVD was not examined, as the uterine size had a minor impact on fertility and was not the object of this study, but this effect has been reported previously

(LeBlanc et al., 2002a). External vaginal discharge was mainly noticed by farmers and is a useful means for a rough estimation of the subsequent pregnancy risk of cows with clinical endometritis.

The microbiological analysis at PC 1 revealed that the presence of TP in the uterus was a crucial factor for the pregnancy outcome at all regarded DIM times, independent of which information was considered. In addition to *E. coli*, TP is known to be a relevant uterine pathogen associated with clinical endometritis that often acts synergistically with other pathogens, such as Gram-negative anaerobes, to enhance the severity of disease (Sheldon et al., 2006, Williams et al., 2005). Similar to the results of previous studies, *E. coli* and TP were the most often identified genera in uterine swabs. Bacterial contamination of the uterus leads to inflammation of the endometrium accompanied by histological damage and delayed uterine involution (Bonnett et al., 1993, Miller et al., 2007, Sheldon et al., 2006, Sheldon et al., 2003). The toxins from bacteria also disturb follicular growth and function (Sheldon et al., 2002, Williams et al., 2007) with adverse effects on reproductive performance, such as a lower overall pregnancy risk, more DO and lower FSCR (Bonnett et al., 1993, Gilbert et al., 2005, Kasimanickam et al., 2004). The density of TP in the uterus is higher in cows with clinical endometritis than in healthy ones (Kaufmann et al., 2010, Machado et al., 2012). If TP was present in our study, an ES 3 was diagnosed more frequently than an ES 1 at PC 1, and overall, more cows with TP suffered from an ES 3 than cows without this type of bacteria. As more cows with EVD suffered from an ES 3 than cows without EVD, it can be presumed that there is a link between vaginal discharge and the amount of pus in the uterine lumen, which is again reflected by the detection of TP (Williams et al., 2005). This may explain the finding that predicting the pregnancy outcomes did not differ substantially regardless of whether microbiological analysis was performed or not.

To the best of our knowledge, this is the first study to show an association between the intrauterine presence of *Serratia spp*. and the diagnosis of a clinical endometritis. *Serratia spp.* is a Gram-negative genera of the family Enterobacteriaceae and is of environmental origin. As the third most commonly diagnosed bacterium in general and the second most common bacterium identified in pure cultures, it seems to play an important role in the pathogenesis of clinical endometritis in the current study population. To date, *Serratia liquefaciens* and *Serratia marcescens* have been mainly described as udder pathogens in cows (Bowman et al., 1986, Wilson et al., 1990), and as a nosocomial bacteria with opportunistic properties, they often cause urinary tract diseases in humans (Mahlen, 2011). Inoculation of *Serratia marescens* into the vaginas of mice for 10 consecutive days led to 100% infertility after subsequent mating due

to the sperm-impairing effects of these microorganisms (Vander and Prabha, 2015). In our study, *Serratia liquefaciens* was mainly isolated from the bacteriological swabs, and no negative effect on any of the fertility parameters could be proven. Therefore, it can be assumed there was an association between *Serratia spp.* and clinical endometritis, but on the basis of our analysis, there was no indication for a negative correlation with fertility in affected cows.

Prediction of pregnancy within 100 DIM in cows without EVD or TP was always associated with the current daily milk yield of the cows. With EVD as the main predictor, the threshold of the milk yield was 43 kg/d for further decisions. For example, cows without EVD and a higher milk yield had a poorer pregnancy outcome than low-producing cows. With TP as the main predictor, the threshold of the milk yield was 34 kg/d; nevertheless, a higher milk yield (> 34 kg/d) led to poorer pregnancy rates. This may confirm that high-producing cows often enter a negative energy balance state, which consequently leads to impaired fertility due to reduced immune function, a higher risk of metabolic disorders (Roche et al., 2009) and subsequent delayed uterine involution and resumption of cyclicity (Sheldon, 2004). This is often combined with poor expression of estrous behavior (Roelofs et al., 2010) or a shorter estrus (6.2 h versus 10.9 h) and standing time and lower serum estradiol concentrations in high-producing cows (≥ 39 kg/day) than in cows with a lower milk yield (Lopez et al., 2004). In the current study, metabolic parameters such as NEFA, BHB or milk protein content were not measured, which only allows presumption of negative energy balance in the few cows that were found to be under-conditioned. However, in addition to clinical endometritis, approximately one-third of the study population suffered from concurrent clinical diseases, such as mastitis or lameness, which occur frequently in high-producing cows and have additional negative effects on fertility (Huszenicza et al., 2005, Melendez et al., 2003). The sum of these events that occur during a cow's puerperium highlight the poor predictive accuracy for early pregnancy outcomes (DIM_{100}). The later in the postpartum period the prognosis is made, the more precise it is, as the critical puerperal period is already completed longer. This promotes the perspective of endeavoring to evade the negative effects of production diseases, for instance, by compliance with a longer voluntary waiting period in high-producing cows under genetic enhancement (Dobson et al., 2007).

CONCLUSIONS

As ES 3 is associated with reduced fertility and EVD was determined to be an indicator of ES 3, it is worth focusing on the history of the cow (as reported by the farmer) to predict its future reproductive performance. In addition to the importance of EVD, microbiological examination of intrauterine fluid is a useful tool for predicting pregnancy status at different times during the postpartum period. *Trueperella pyogenes* was identified to be decisive, but the predictive accuracy was similar to prediction based only on anamnestic and clinical information. Consequently, the presence of TP in the uterus is a suitable predictor of the pregnancy outcomes of cows with clinical endometritis, but microbiological analysis is not necessarily needed if the cows are checked regularly for EVD prior to 22 DIM.

ACKNOWLEDGMENTS

The study was funded by the Federal Food Safety and Veterinary Office (FSVO) of Switzerland. There was no financial support provided by pharmaceutical companies or other sponsors. We are grateful to all the participating dairy producers for their invested time and appreciate their specific interest in this topic. Our special thanks to the team of veterinarians of the Ambulatory Service of the Vetsuisse Faculty of Zurich for their support in routine activities and for providing the sample material and the vehicles.

Table 1. Definitions of parameters for quantification of reproductive performance

Parameter	Definition	
DFS = Days to first service (d)	Number of days from calving to first AI	
DO = Days open (d)	Number of days from calving to successful AI	
CRI = Culling rate due to infertility (%)	Cows culled due to infertility / Number of cows in the study population (N)	x 100
FSCR = First service conception rate (%)	Cows pregnant to first AI / Number of cows inseminated	x 100
CRAS = Conception rate to all services (%)	Total number of cows pregnant / Total number of AI	x 100
TCR = Total conception rate (%)	Number of cows pregnant / Number of cows inseminated	x 100

Table 2. Univariate analysis of the effects of risk factors on the endometritis score (ES)[1] at puerperal control 1 (PC 1 = 22-45 DIM). Data from 300 dairy cows (200 without CL, 100 with CL) were analyzed

Variable	Category	n	Endometritis score (ES) at PC1		
			1	2	3
Parity	Primiparous	77	20 (26%)^{a}	36 (47%)^{b}	21 (27%)^{a}
	Multiparous	223	85 (38%)^{a}	84 (38%)^{a}	54 (24%)^{b}
BCS[2]	Under-conditioned	35	13 (37%)	12 (34%)^{x,y}	10 (29%)
	Adequate condition	226	82 (36%)^{a}	86 (38%)^{a,x}	58 (26%)^{b}
	Over-conditioned	39	10 (26%)^{a}	22 (56%)^{b,y}	7 (18%)^{a}
Puerperal disease	Yes	102	26 (26%)^{a,x}	40 (39%)^{b}	36 (35%)^{a,b,x*}
(within the first 3 weeks p.p.)	No	198	79 (40%)^{a,y}	80 (40%)^{a}	39 (20%)^{b,y*}
External vaginal	Yes	104	21 (20%) ^{a,x*}	42 (40%)^{b}	41 (39%)^{b,x*}
Discharge	No	196	84 (43%) ^{a,y*}	79 (40%)^{a}	34 (17%)^{b,y*}
Daily milk yield	15 – 30 kg	114	50 (35%) ^{a,b}	65 (45%)^{a,x*}	29 (20%)^{b}
	31 – 45 kg	128	41 (32%)	50 (39%)^{x}	37 (29%)
	46 – 60 kg	28	14 (50%)^{a}	5 (18%)^{b,y*}	9 (32%)^{a,b}
Concurrent diseases	Yes	89	74 (35%)^{a,b}	87 (41%)^{a}	50 (24%)^{b}
	No	211	31 (35%)^{a,b}	33 (37%)^{a}	25 (28%)^{b}
Corpus luteum (CL)	Yes	100	28 (28%)	38 (38%)	34 (34%)^{x}
at PC1	No	200	77 (39%)^{a*}	82 (41%)^{a*}	41 (21%)^{b*,y}
Type of treatment	GnRH (no CL)	100	47 (47%)^{a*,x}	38 (38%)^{a*}	15 (15%)^{b*,x*}
	Antibiotics (no CL)	100	30 (30%)^{a,y}	44 (44%)^{b}	26 (26%)^{a,y}
	Prostaglandin $F_{2\alpha}$ (CL)	100	28 (28%)^{y}	38 (38%)	34 (34%)^{y*}
Uterine size[3]	1	58	20 (35%)^{a}	28 (48%)^{a*}	10 (17%)^{b*,x}
	2	167	63 (38%)^{a*}	66 (39%)^{a*}	38 (23%)^{b*,x}
	3	75	22 (29%)	26 (35%)	27 (36%)^{y}
Odor of vaginal	Fetid	75	4 (5%)^{a*,x*}	25 (33%)^{b*}	46 (61%)^{c*,x*}
Discharge	Neutral	225	101 (45%)^{a*,y*}	95 (42%)^{a*}	29 (13%)^{b*,y*}
Uterine fluctuation	Yes	37	6 (16%)$^{a*.x*}$	17 (46%)^{b*}	14 (38%)^{b,x}
	No	263	99 (38%)^{a*,y*}	103 (39%)^{a*}	61 (23%)^{b*,y}
Ultrasonographically	Yes	253	92 (36%)^{a*}	103 (42%)^{a*}	58 (23%)^{b*}
visible uterine content	No	45	13 (28%)	16 (36%)	16 (36%)
Cervical diameter	< 5 cm	146	58 (40%)^{a*}	62 (43%)^{a*}	26 (18%)^{b*,x*}
	≥ 5 cm	154	47 (31%)	58 (38%)	49 (32%)^{y*}
T. pyogenes	Yes	90	16 (18%)^{a*,x*}	29 (32%)^{b}	45 (50%)^{c*,x*}
	No	206	88 (43%)^{a*,y*}	88 (43%)^{a*}	30 (14%)^{b*,y*}

[1] Endometritis scores (ES): ES 1 = mucus with flecks of pus, ES 2 = vaginal discharge containing less than 50% mucopurulent material, ES 3 = mucus with more than 50% purulent discharge.

[2] BCS for (Holstein Friesian, Red Holstein, Brown Swiss)/Simmental:
Under-conditioned = < 2.5/3, adequate condition = 2.5/3 – 3.25/3.75, over-conditioned = > 3.25/3.75.

[3] size 1 = uterus delimitable by hand, size 2 = uterus nearly delimitable, size 3 = uterus undelimitable and mainly situated outside the pelvic cavity.

^{a,b,c} Within rows, values with different superscripts differ significantly (P-value < 0.05) between ES 1, ES 2 and ES 3.

^{x,y} Within columns, values with different superscripts differ significantly (P-value < 0.05) between the subsets of the variables.

* $P < 0.01$.

Table 3. Odds ratio of the risk factors for an endometritis score 3 (ES 3) at puerperal control 1 (PC1 = 22-45 DIM) with < ES 3 as reference

Variable	Category	% of cows with ES 3 at PC 1	OR[1]	95 % CI	*P*-value
Puerperal disease within the first 3 weeks p.p.[2]	Yes	35	2.22	1.30 – 3.80	< 0.01
	No	20	Reference		
External vaginal discharge	Yes	39	3.10	1.81 – 5.32	< 0.01
	No	17	Reference		
Presence of a CL[3] at PC1	Yes	34	2.00	1.17 – 3.42	0.01
	No	21	Reference		
Odor of vaginal discharge	Fetid	61	10.7	5.85 – 19.67	< 0.01
	Neutral	13	Reference		
Uterine fluctuation	Yes	38	2.02	0.98 – 4.16	0.05
	No	23	Reference		
Cervical diameter	≥ 5 cm	18	2.15	1.25 – 3.71	0.01
	< 5 cm	32	Reference		
Trueperella pyogenes	Yes	50	5.87	3.33 – 10.34	< 0.01
	No	15	Reference		

[1] Odds ratio.

[2] Postpartum.

[3] Corpus luteum.

Table 4. Univariate effects of single risk factors based on anamnestic information or cow-specific data on reproduction performance parameters: days to first service (DFS), days open (DO), first service conception rate (FSCR), conception rate to all services (CRAS) and culling rate due to infertility (CRI)

				DFS		**DO**		**FSCR**		**CRAS**		**CRI**	
		N[1]	%	n[2]	mean ± SD	n	mean ± SD	n	%	n	%	n	%
Anamnestic findings	total	300	100										
Parity	Primiparous	77	26	72	76 ± 24	58	103 ± 41	31	43	58	75	14	18
	Multiparous	223	74	206	81 ± 27	148	107 ± 41	69	33	148	66	57	26
Body condition score (BCS)	Under-conditioned	35	12	32	80 ± 27	25	107 ± 43^{a,b}	11	34^{a,b}	25	38	4	11^{a}
	Adequate condition	226	75	210	80 ± 26	156	109 ± 40^{a}	70	33^{a}	156	34	54	24^{a,b}
	Over-conditioned	39	13	36	77 ± 27	24	88 ± 37^{b}	19	53^{b}	24	31	13	33^{b}
Puerperal disease[3]	Yes	102	34	91	82 ± 29	60	116 ± 41^{a}	24	26^{a}	60	28^{a}	31	30
	No	198	66	188	78 ± 25	146	103 ± 40^{b}	76	40^{b}	146	38^{b}	40	20
Concurrent disease	Mastitis	38	13	32	84 ± 31	21	127 ± 44	7	22^{a,c}	21	27^{a}*^{,c}	9	24^{a,b}
	Claw disorders	24	8	20	85 ± 30	17	98 ± 32	11	55^{b}*	17	29^{b}*	2	8^{a}
	Others	27	9	25	84 ± 31	13	120 ± 47	4	25^{c}*	13	22^{a}*	10	37^{b}
	None	211	70	202	78 ± 25	155	103 ± 40	78	39^{a,b}	155	36^{c}	50	24^{a,b}
External vaginal discharge	Yes	104	35	92	79 ± 23	58	116 ± 42	21	23^{a}*	58	25^{a}*	35	34^{a}*
	No	196	65	186	80 ± 28	147	102 ± 40	79	41^{b}*	147	40^{b}*	36	18^{b}*
Daily milk yield	15 – 30 kg	144	48	134	75 ± 24	104	100 ± 43^{a}	57	40^{a}*	104	38^{a}	33	23
	31 – 45 kg	128	43	120	83 ± 29	85	111 ± 37^{a,b}	39	30^{a,b}	85	33^{a,b}	30	23
	46 – 60 kg	28	9	25	86 ± 24	17	125 ± 40^{b}	4	14^{b}*	17	25^{b}	8	29

[1]N = total number of cows in the subgroups.
[2]n = number of cows which could be considered for the calculation of the respective reproduction performance parameter.
[3]Cows with a history of a puerperal disease such as retained fetal membranes or metritis.
a,b,c Within columns, values with different superscripts differ significantly ($P < 0.05$).
*$P < 0.01$.

Table 5. Univariate analysis of single risk factors based on the type of treatment and examination data of Puerperal control 1 (PC 1) at 22 – 45 DIM on reproduction performance parameters: days to first service (DFS), days open (DO), first service conception rate (FSCR), conception rate to all services (CRAS) and culling rate due to infertility (CRI)

				DFS		**DO**		**FSCR**		**CRAS**		**CRI**	
		N[1]	%	n[2]	mean ± SD	n	mean ± SD	n	%	n	%	n	%
Puerperal control 1	total	300	100										
Type of treatment	GnRH (no CL)	100	33	94	79 ± 27	71	112 ± 42	31	33	71	33	20	20
	Antibiotics (no CL)	100	33	94	80 ± 29	67	104 ± 40	31	33	67	33	26	26
	Prostaglandin F2α (CL)	100	33	91	80 ± 24	68	103 ± 40	38	42	68	37	25	25
CL	Yes	100	33	91	80 ± 24	68	103 ± 40	38	42	68	37	25	25
	No	200	66	188	79 ± 28	138	108 ± 41	62	33	138	33	46	23
Uterine size	1	58	19	56	78 ± 25	43	104 ± 36	20	$36^{a,b}$	43	38^{a}	11	19^{a}
	2	167	56	154	80 ± 26	121	107 ± 41	63	41^{a}	121	37^{a}	32	19^{a}*
	3	75	25	68	81 ± 28	41	107 ± 45	17	25^{b}	41	25^{b}	28	37^{b}*
EM discharge score	1	105	35	99	$79 \pm 26^{a,b}$	78	108 ± 40	40	40	78	$75^{a,b}$	22	$21^{a,b}$
	2	120	40	114	77 ± 26^{a}	88	101 ± 40	42	37	88	73^{a}	24	20^{a}
	3	75	25	65	86 ± 26^{b}	39	114 ± 44	18	28	39	52^{b}	25	33^{b}
Odor of vaginal	Fetid	75	25	66	84 ± 27	44	111 ± 46	20	30	44	59	49	22
Discharge	Neutral	225	75	213	78 ± 26	161	105 ± 39	80	38	161	72	22	29
Fluctuation of uterine	Yes	37	12	36	69 ± 24^{a}*	24	100 ± 49	11	31	24	26	11	30
Content	No	263	88	243	81 ± 27^{b}*	182	107 ± 39	89	37	182	36	60	23
Visible uterine	Yes	253	84	235	79 ± 26	171	107 ± 42	82	35	171	33	63	25
content (ultrasound)	No	45	16	42	84 ± 31	33	103 ± 38	18	43	33	41	8	45
Cervical diameter	< 5 cm	154	51	136	81 ± 28	103	106 ± 41	47	35	103	36	32	21
	≥ 5 cm	146	49	143	78 ± 25	103	107 ± 40	53	37	103	33	39	27
T. pyogenes[3]	Yes	90	30	79	86 ± 31^{a}	49	125 ± 46^{a}*	16	20^{a}*	49	26^{a}*	28	31^{a}*
	No	206	70	197	77 ± 24^{b}	156	100 ± 37^{b}*	84	43^{b}*	156	39^{b}*	41	20^{b}*

[1]N = total number of cows in the subgroups.

[2]n = number of cows which could be considered for the calculation of the respective reproduction performance parameter.

[3]Note: Other bacteria than *T. pyogenes* (*E. coli*, *Serratia spp*., *CNS*, *Streptococcus spp*., *Enterococcus*, Gram-neg. Anaerobes, *Klebsiella*, Others) did not have any effect on the reproduction performance parameters and were therefore not listed in the table.

a,b Within columns, values with different superscripts differ significantly ($P < 0.05$).

*$P < 0.01$.

Table 6. Univariate analysis of single risk factors based on data of Puerperal control 2 (PC 2) at 43 – 66 DIM (21 days later than PC 1) on reproduction performance parameters: days to first service (DFS), days open (DO), first service conception rate (FSCR), conception rate to all services (CRAS) and culling rate due to infertility (CRI)

				DFS		**DO**		**FSCR**		**CRAS**		**CRI**	
		N[1]	%	n[2]	mean ± SD	n	mean ± SD	n	%	n	%	n	%
Puerperal control 2	total	298	100										
Endometritis discharge	0	160	54	154	77 ± 27[a]	116	102 ± 40	61	40[a]	116	35[a]	36	23[a]*
Score	1	91	30	87	80 ± 26[a,b]	65	108 ± 41	30	34[a,b]	65	35[a]	20	22[a]*
	2	26	9	22	86 ± 24[b]	19	116 ± 41	7	32[a,b]	19	45[a]*	2	8[a]*
	3	21	7	16	91 ± 28[b]	6	136 ± 44	2	13[b]	6	15[b]*	12	57[b]*
Odor of vaginal discharge	Fetid	20	7	16	91 ± 31	10	131 ± 47	3	19	10	28	7	35
	Neutral	278	93	263	79 ± 26	196	105 ± 40	97	37	196	35	63	23
Ovarian findings	CL	159	53	152	79 ± 28	118	105 ± 43	58	38[a]	118	37[a]	33	21
	Dominant follicle	71	24	66	78 ± 24	44	114 ± 37	15	23[b]	44	27[b]	21	30
	No ovarian activity	42	14	36	91 ± 31	25	105 ± 45	18	50[a]	25	37[a,b]	11	26
	Follicle-theca-cyst	26	8	25	71 ± 11	19	95 ± 26	9	36[a,b]	19	36[a,b]	5	19

[1]N = total number of cows in the subgroups.

[2]n = number of cows which could be considered for the calculation of the respective reproduction performance parameter.

a,b Within columns, values with different superscripts differ significantly ($P < 0.05$).

*$P < 0.01$.

Table 7. Conditional inference trees for the pregnancy rate at different days in milk (DIM_{100}, DIM_{150}, DIM_{I00}) in consideration of selected combinations of examination results. Percentage (%) of cows pregnant is listed dependent on the presence of the predictor variables. Only significant results are shown ($P < 0.01$)

Considered data in the statistical model	DIM_{100}	DIM_{150}	DIM_{200}
PC 1*	External vaginal discharge Yes (n = 104) / No (n = 196) Current daily milk yield 22 % / ≤ 43 kg (n = 169) / > 43 kg (n = 27) 47 % / 11 %	External vaginal discharge Yes (n = 104) / No (n = 196) 42 % / 65 %	External vaginal discharge Yes (n = 104) / No (n = 196) 58 % / 76 %
PC 1 + ultrasonography**	External vaginal discharge Yes (n = 104) / No (n = 196) Current daily milk yield 22 % / ≤ 43 kg (n = 169) / > 43 kg (n = 27) 47 % / 11 %	External vaginal discharge Yes (n = 104) / No (n = 196) 42 % / 65 %	External vaginal discharge Yes (n = 104) / No (n = 196) 58 % / 76 %
PC 1 + microbiology***	*T. pyogenes* Yes (n = 90) / No (n = 196) Current daily milk yield 20 % / ≤ 34 kg (n = 121) / > 34 kg (n = 89) 52 % / 28 %	*T. pyogenes* Yes (n = 90) / No (n = 210) 38 % / 66 %	*T. pyogenes* Yes (n = 90) / No (n = 210) 54 % / 75 %
PC 1 + PC 2****	External vaginal discharge Yes (n = 104) / No (n = 196) Current daily milk yield 22 % / ≤ 43 kg (n = 169) / > 43 kg (n = 27) 47 % / 11 %	External vaginal discharge Yes (n = 104) / No (196) ES day 21 42 % / ≤ 2 (n = 186) / > 2 (n = 10) 68 % / 10 %	External vaginal discharge Yes (n = 104) / No (196) ES day 21 58 % / ≤ 2 (n = 186) / > 2 (n = 10) 78 % / 20 %
All available data	*T. pyogenes* Yes (n =90) / No (n = 210) Current daily milk yield 20 % / ≤ 34 kg (n = 121) / > 34 kg (n = 89) 51 % ES day 21 0 (n = 57) / > 0 (n = 32) 40 % / 6 %	*T. pyogenes* Yes (n = 90) / No (n = 210) 38 % / 66 %	*T. pyogenes* Yes (n = 90) / No (n = 210) 54 % / 75 %
Probability to predict Pregnancy[1]	Yes / 25% / No / 85 %	Yes / 70 % / No / 40 %	Yes / 90 % / No / 20 %

*data of PC 1 (puerperal control 1, 22-45 DIM): parity, current daily milk yield, puerperal diseases prior to the study, concurrent diseases, BCS, external vaginal discharge, type of treatment, endometritis score (ES) at PC 1, odor of the vaginal discharge at PC 1, uterine size.
**ultrasonography: any visible uterine content, cervical diameter.
***microbiology: *E. coli*, *T. pyogenes*, *Serratia spp*., *CNS*, *Streptococcus spp*., *Enterococcus spp.*, Gram-neg. Anaerobes, *Klebsiella spp.*, Others.
****data of PC 2 (puerperal control 2, 21 ± 1 days after PC 1): odor of vaginal discharge at PC 2, ES at PC 2, ovarian findings at PC 2.
[1]random forests with the same predictors were used to assess the probability to predict the pregnancy outcome. The percentages apply to all models of the column above.

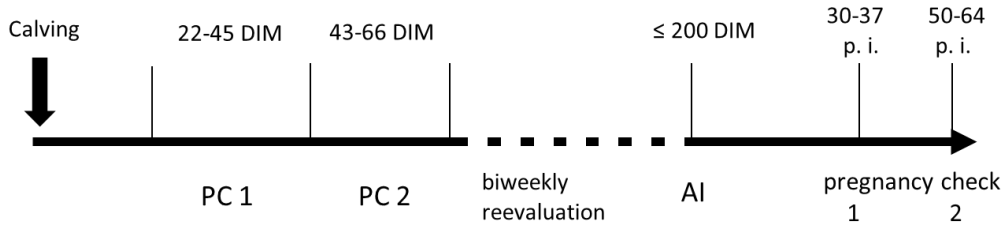

Figure 1. Study schedule for 300 cows with clinical endometritis (200 cows without a corpus luteum (CL), 100 cows with CL), which were included in the study at 22-45 DIM and followed until 200 DIM (AI at 200 DIM at the latest)
PC 1 = Puerperal control 1: Metricheck, gynecological examination (including ultrasonography), microbiology, treatment
PC 2 = Puerperal control 2: Metricheck, gynecological examination (including ultrasonography), treatment
Biweekly reevaluation: regular gynecological examinations until ES 0 was achieved and thus the cows were ready for AI
Abbreviations: AI = artrtificial insemination, p.i. = post insemination

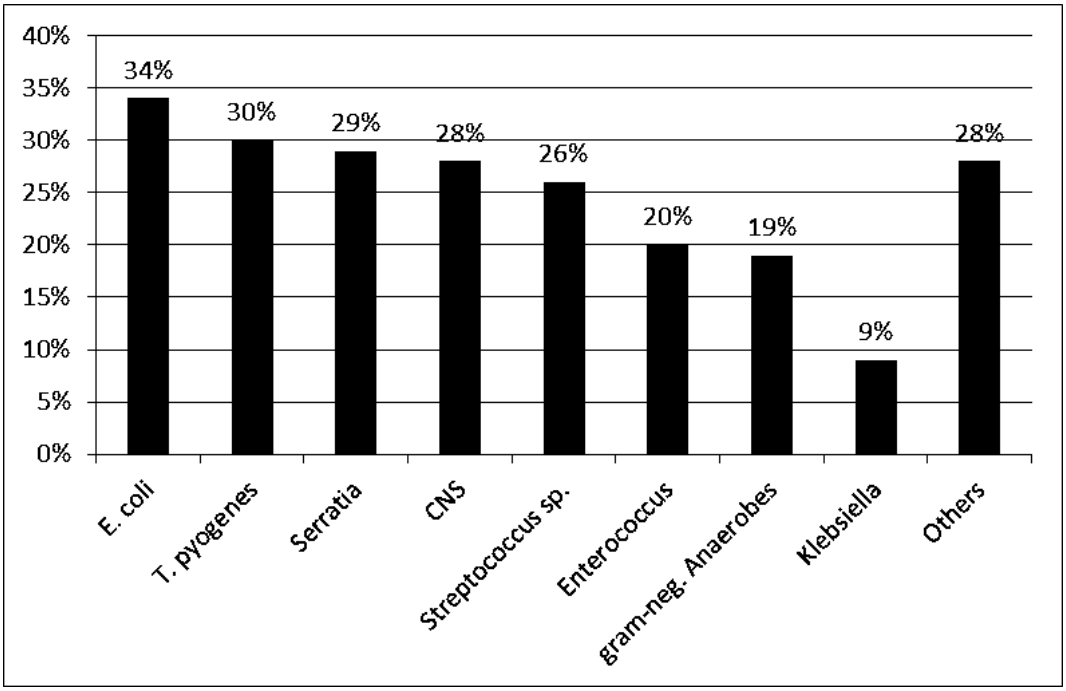

Figure 2. Proportions of bacteria (%) isolated from the uterine lumen by bacteriological culture of 296 intrauterine swabs at Puerperal control 1 (22-45 DIM)
Note: The total amount exceeds 100% due to the frequent occurrence of mixed cultures.

REFERENCES

Barlund, C. S., T. D. Carruthers, C. L. Waldner, and C. W. Palmer. 2008. A comparison of diagnostic techniques for postpartum endometritis in dairy cattle. Theriogenology 69(6):714-723.

Bekana, M., P. Jonsson, T. Ekman, and H. Kindahl. 1994. Intrauterine Bacterial Findings in Postpartum Cows with Retained Fetal Membranes. Journal of Veterinary Medicine Series a-Zentralblatt Fur Veterinarmedizin Reihe a-Physiology Pathology Clinical Medicine 41(9):663-670.

Bicalho, M. L. S., F. S. Lima, V. S. Machado, E. B. Meira, E. K. Ganda, C. Foditsch, R. C. Bicalho, and R. O. Gilbert. 2016. Associations among Trueperella pyogenes, endometritis diagnosis, and pregnancy outcomes in dairy cows. Theriogenology 85(2):267-274.

Bonnett, B. N., S. W. Martin, and A. H. Meek. 1993. Associations of Clinical Findings, Bacteriological and Histological Results of Endometrial Biopsy with Reproductive-Performance of Postpartum Dairy-Cows. Prev Vet Med 15(2-3):205-220.

Bowman, G. L., W. D. Hueston, G. J. Boner, J. J. Hurley, and J. E. Andreas. 1986. Serratia liquefaciens mastitis in a dairy herd. J. Am. Vet. Med. Assoc. 189(8):913-915.

Denis-Robichaud, J. and J. Dubuc. 2015. Determination of optimal diagnostic criteria for purulent vaginal discharge and cytological endometritis in dairy cows. J. Dairy Sci. 98(10):6848-6855.

Dobson, H., R. F. Smith, M. D. Royal, C. H. Knight, and I. M. Sheldon. 2007. The high-producing dairy cow and its reproductive performance. Reproduction in Domestic Animals 42:17-23.

Drillich, M., D. Raab, M. Wittke, and W. Heuwieser. 2005. Treatment of chronic endometritis in dairy cows with an intrauterine application of enzymes. A field trial. Theriogenology 63(7):1811-1823.

Dubuc, J., T. F. Duffield, K. E. Leslie, J. S. Walton, and S. J. LeBlanc. 2010. Definitions and diagnosis of postpartum endometritis in dairy cows. J. Dairy Sci. 93(11):5225-5233.

Dubuc, J., T. F. Duffield, K. E. Leslie, J. S. Walton, and S. J. LeBlanc. 2012. Risk factors and effects of postpartum anovulation in dairy cows. J. Dairy Sci. 95(4):1845-1854.

Foldi, J., M. Kulcsar, A. Pecsi, B. Huyghe, C. de Sa, J. A. Lohuis, P. Cox, and G. Huszenicza. 2006. Bacterial complications of postpartum uterine involution in cattle. Anim Reprod Sci 96(3-4):265-281.

Fonseca, F. A., J. H. Britt, B. T. McDaniel, J. C. Wilk, and A. H. Rakes. 1983. Reproductive traits of Holsteins and Jerseys. Effects of age, milk yield, and clinical abnormalities on involution of cervix and uterus, ovulation, estrous cycles, detection of estrus, conception rate, and days open. J. Dairy Sci. 66(5):1128-1147.

Gautam, G., T. Nakao, M. Yusuf, and K. Koike. 2009. Prevalence of endometritis during the postpartum period and its impact on subsequent reproductive performance in two Japanese dairy herds. Anim Reprod Sci 116(3-4):175-187.

Gier, H. T. and G. B. Marion. 1968. Uterus of the cow after parturition: involutional changes. American Journal of Veterinary Research 29(1):83-96.

Gilbert, R. O., S. T. Shin, C. L. Guard, H. N. Erb, and M. Frajblat. 2005. Prevalence of endometritis and its effects on reproductive performance of dairy cows. Theriogenology 64(9):1879-1888.

Gobikrushanth, M., R. Salehi, D. J. Ambrose, and M. G. Colazo. 2016. Categorization of endometritis and its association with ovarian follicular growth and ovulation, reproductive performance, dry matter intake, and milk yield in dairy cattle. Theriogenology 86(7):1842-1849.

Grohn, Y. T. and P. J. Rajala-Schultz. 2000. Epidemiology of reproductive performance in dairy cows. Anim Reprod Sci 60-61:605-614.

Hendricks, K. E., J. A. Bartolome, P. Melendez, C. Risco, and L. F. Archbald. 2006. Effect of repeated administration of PGF2alpha in the early post partum period on the prevalence of clinical endometritis and probability of pregnancy at first insemination in lactating dairy cows. Theriogenology 65(8):1454-1464.

Heuwieser, W. and R. Mansfeld. 1992. Beurteilung der Körperkondition bei Milchkühen. Teil 2. Milchpraxis 30:10-14.

Hothorn, T., K. Hornik, and A. Zeileis. 2006. Unbiased recursive partitioning: A conditional inference framework. J Comput Graph Stat 15(3):651-674.

Hothorn, T. and A. Zeileis. 2015. partykit: A Modular Toolkit for Recursive Partytioning in R. J Mach Learn Res 16:3905-3909.

Huszenicza, G., S. Janosi, M. Kulcsar, P. Korodi, J. Reiczigel, L. Katai, A. R. Peters, and F. De Rensis. 2005. Effects of clinical mastitis on ovarian function in post-partum dairy cows. Reprod. Domest. Anim. 40(3):199-204.

Kasimanickam, R., T. F. Duffield, R. A. Foster, C. J. Gartley, K. E. Leslie, J. S. Walton, and W. H. Johnson. 2004. Endometrial cytology and ultrasonography for the detection of subclinical endometritis in postpartum dairy cows. Theriogenology 62(1-2):9-23.

Kaufmann, T. B., S. Westermann, M. Drillich, J. Plontzke, and W. Heuwieser. 2010. Systemic antibiotic treatment of clinical endometritis in dairy cows with ceftiofur or two doses of cloprostenol in a 14-d interval. Anim Reprod Sci 121(1-2):55-62.

Kim, I. H. and H. G. Kang. 2003. Risk factors for postpartum endometritis and the effect of endometritis on reproductive performance in dairy cows in Korea. J. Reprod. Dev. 49(6):485-491.

Knutti, B., U. Kupfer, and A. Busato. 2000. Reproductive efficiency of cows with endometritis after treatment with intrauterine infusions or prostaglandin injections, or no treatment. J Vet Med A Physiol Pathol Clin Med 47(10):609-615.

LeBlanc, S. J., T. F. Duffield, K. E. Leslie, K. G. Bateman, G. P. Keefe, J. S. Walton, and W. H. Johnson. 2002a. Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. J. Dairy Sci. 85(9):2223-2236.

LeBlanc, S. J., T. F. Duffield, K. E. Leslie, K. G. Bateman, G. P. Keefe, J. S. Walton, and W. H. Johnson. 2002b. The effect of treatment of clinical endometritis on reproductive performance in dairy cows. J. Dairy Sci. 85(9):2237-2249.

Lopez-Helguera, I., F. Lopez-Gatius, and I. Garcia-Ispierto. 2012. The influence of genital tract status in postpartum period on the subsequent reproductive performance in high producing dairy cows. Theriogenology 77(7):1334-1342.

Lopez, H., L. D. Satter, and M. C. Wiltbank. 2004. Relationship between level of milk production and estrous behavior of lactating dairy cows. Anim Reprod Sci 81(3-4):209-223.

Machado, V. S., W. A. Knauer, M. L. Bicalho, G. Oikonomou, R. O. Gilbert, and R. C. Bicalho. 2012. A novel diagnostic technique to determine uterine health of Holstein cows at 35 days postpartum. J. Dairy Sci. 95(3):1349-1357.

Mahlen, S. D. 2011. Serratia infections: from military experiments to current practice. Clin. Microbiol. Rev. 24(4):755-791.

McDougall, S. 2001. Effect of intrauterine antibiotic treatment on reproductive performance of dairy cows following periparturient disease. N Z Vet J 49(4):150-158.

McDougall, S., H. Hussein, D. Aberdein, K. Buckle, J. Roche, C. Burke, M. Mitchell, and S. Meier. 2011. Relationships between cytology, bacteriology and vaginal discharge scores and reproductive performance in dairy cattle. Theriogenology 76(2):229-240.

McDougall, S., R. Macaulay, and C. Compton. 2007. Association between endometritis diagnosis using a novel intravaginal device and reproductive performance in dairy cattle. Anim Reprod Sci 99(1-2):9-23.

Melendez, P., J. Bartolome, L. F. Archbald, and A. Donovan. 2003. The association between lameness, ovarian cysts and fertility in lactating dairy cows. Theriogenology 59(3-4):927-937.

Miller, A. N., E. J. Williams, K. Sibley, S. Herath, E. A. Lane, J. Fishwick, D. M. Nash, A. N. Rycroft, H. Dobson, C. E. Bryant, and I. M. Sheldon. 2007. The effects of Arcanobacterium pyogenes on endometrial function in vitro, and on uterine and ovarian function in vivo. Theriogenology 68(7):972-980.

Pleticha, S., M. Drillich, and W. Heuwieser. 2009. Evaluation of the Metricheck device and the gloved hand for the diagnosis of clinical endometritis in dairy cows. J. Dairy Sci. 92(11):5429-5435.

Roche, J. R., N. C. Friggens, J. K. Kay, M. W. Fisher, K. J. Stafford, and D. P. Berry. 2009. Invited review: Body condition score and its association with dairy cow productivity, health, and welfare. J. Dairy Sci. 92(12):5769-5801.

Roelofs, J., F. Lopez-Gatius, R. H. Hunter, F. J. van Eerdenburg, and C. Hanzen. 2010. When is a cow in estrus? Clinical and practical aspects. Theriogenology 74(3):327-344.

Ruder, C. A., R. G. Sasser, R. J. Williams, J. K. Ely, R. C. Bull, and J. E. Butler. 1981. Uterine Infections in the Postpartum Cow .1. Effect of Dietary Crude Protein Restriction. Theriogenology 15(6):561-572.

Runciman, D. J., G. A. Anderson, and J. Malmo. 2009. Comparison of two methods of detecting purulent vaginal discharge in postpartum dairy cows and effect of intrauterine cephapirin on reproductive performance. Aust. Vet. J. 87(9):369-378.

Runciman, D. J., G. A. Anderson, J. Malmo, and G. M. Davis. 2008. Use of postpartum vaginoscopic (visual vaginal) examination of dairy cows for the diagnosis of endometritis and the association of endrometritis with reduced reproductive performance. Aust. Vet. J. 86(6):205-213.

Senosy, W. S., M. Uchiza, N. Tameoka, Y. Izaike, and T. Osawa. 2009. Association between evaluation of the reproductive tract by various diagnostic tests and restoration of ovarian cyclicity in high-producing dairy cows. Theriogenology 72(9):1153-1162.

Sheldon, I. M. 2004. The postpartum uterus. Vet. Clin. North Am. Food Anim. Pract. 20(3):569-591.

Sheldon, I. M., G. S. Lewis, S. LeBlanc, and R. O. Gilbert. 2006. Defining postpartum uterine disease in cattle. Theriogenology 65(8):1516-1530.

Sheldon, I. M., D. E. Noakes, A. N. Rycroft, and H. Dobson. 2003. The effect of intrauterine administration of estradiol on postpartum uterine involution in cattle. Theriogenology 59(5-6):1357-1371.

Sheldon, I. M., D. E. Noakes, A. N. Rycroft, D. U. Pfeiffer, and H. Dobson. 2002. Influence of uterine bacterial contamination after parturition on ovarian dominant follicle selection and follicle growth and function in cattle. Reproduction 123(6):837-845.

Vander, H. and V. Prabha. 2015. Evaluation of fertility outcome as a consequence of intravaginal inoculation with sperm-impairing micro-organisms in a mouse model. Journal of Medical Microbiology 64:344-347.

Westermann, S., M. Drillich, T. B. Kaufmann, L. V. Madoz, and W. Heuwieser. 2010. A clinical approach to determine false positive findings of clinical endometritis by vaginoscopy by the use of uterine bacteriology and cytology in dairy cows. Theriogenology 74(7):1248-1255.

Williams, E. J., D. P. Fischer, D. E. Noakes, G. C. England, A. Rycroft, H. Dobson, and I. M. Sheldon. 2007. The relationship between uterine pathogen growth density and ovarian function in the postpartum dairy cow. Theriogenology 68(4):549-559.

Williams, E. J., D. P. Fischer, D. U. Pfeiffer, G. C. England, D. E. Noakes, H. Dobson, and I. M. Sheldon. 2005. Clinical evaluation of postpartum vaginal mucus reflects uterine bacterial infection and the immune response in cattle. Theriogenology 63(1):102-117.

Wilson, D. J., J. H. Kirk, R. D. Walker, and Q. W. Bosworth. 1990. Serratia marcescens mastitis in a dairy herd. J. Am. Vet. Med. Assoc. 196(7):1102-1105.

CURRICULUM VITAE

Vorname Name	Martina Ernstberger
Geburtsdatum	29.06.1987
Geburtsort	95643 Tirschenreuth, Deutschland
Nationalität	Deutsch

September/1997 – Juni/2006	**Stiftland Gymnasium, 95643 Tirschenreuth, Deutschland**
30.06.2006	Abitur, Stiftland Gymnasium, 95643 Tirschenreuth, Deutschland
Oktober/2007 – Dezember/2013	**Studium der Veterinärmedizin, Veterinärmedizinische Universität Wien, 1210 Wien, Österreich**
09.12.2013	Abschluss der Diplomarbeit, Veterinärmedizinische Universität Wien, 1210 Wien, Österreich
Oktober/2014 – August/2018	**Anfertigung der Dissertation** Unter Leitung von Prof. Dr. Heinrich Bollwein am Departement für Nutztiere der Vetsuisse-Fakultät, Universität Zürich Direktor Prof. Dr. Heinrich Bollwein

Fachrelevante Anstellungen nach Abschluss des veterinärmedizinischen Studiums

Oktober/2013 – Juli/2014	**Assistenzärztin**, Stationäre Klinik der Wiederkäuerklinik Bern, Vetsuisse Fakultät Universität Bern, 3012 Bern, Schweiz
September/2014 – heute	**Assistenzärztin**, Ambulanz und Bestandesmedizin, Departement für Nutztiere, Vetsuisse Fakultät Universität Zürich, 8057 Zürich, Schweiz